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AMIF RESEARCH REPORT

REGARDING:

Meat Pigments

Fats

Nutrition

Meat By-Products

Pork Tongue Abscesses

Meat Processing

Meat Preservation

Meat Tenderness

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AMERICAN MEAT INSTITUTE FOUNDATION

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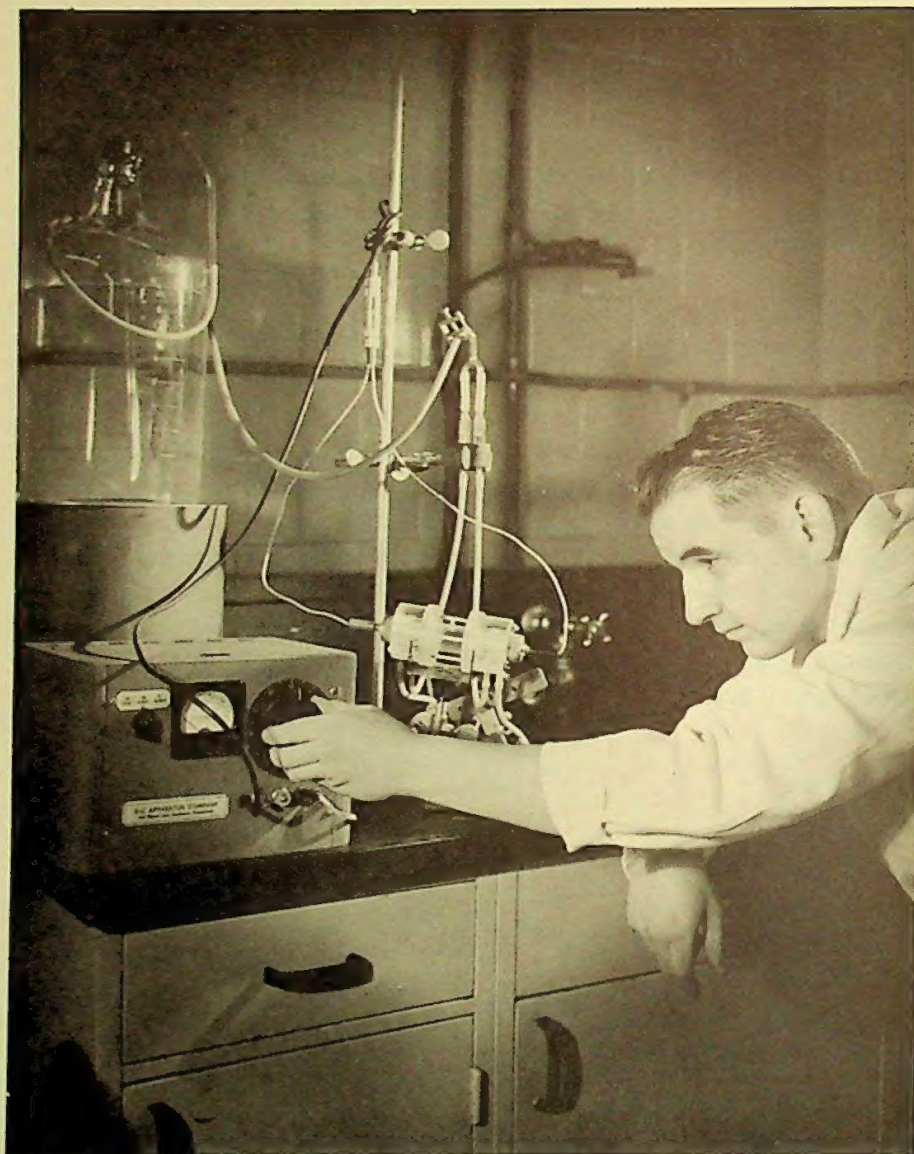
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FOREWORD

In this publication, the American Meat Institute Foundation presents concise summaries of reports regarding twenty AMIF research projects. These reports were presented by staff members during a special meeting held in Chicago on March 26, 1958, for representatives of establishments participating in the support of Foundation research.

It should be noted that, in some cases, research discussed had not been completed at the time of the meeting. In such cases, no conclusions or only tentative conclusions were outlined. Detailed information regarding the results of AMIF research is, of course, published by the Foundation as studies are completed or as the research reaches a stage where constructive data have become available.



A salt-free sample of meat pigment (myoglobin) is prepared by Dr. J. B. Fox, Jr., of Biochemistry and Nutrition during one of several phases of study of the chemistry of meat color.

MEAT PIGMENTS AND FATS

Studies on the Physical and Chemical Characteristics of Myoglobin

By J. B. Fox, Jr.

Division of Biochemistry and Nutrition

Previous meat pigment studies have been concerned with the basic chemical characteristics of myoglobin and with the changes that occur during the processing, preservation, and storage of meat, with emphasis on the color changes during irradiation preservation. These latter studies have indicated that the red pigment of irradiated meat is either an altered form of oxymyoglobin or a new type of heme pigment complex.

Observations on the pink pigments in cooked uncured meats and the time-temperature relationships required to produce cured meat pigments have long indicated the need for understanding the basic chemical and physical changes that occur when whole meats are subjected to elevated temperatures. Investigations into the rate of thermal denaturation of the muscle heme pigment, myoglobin, have shown that the protein moiety is remarkably stable to heat. The rate of denaturation at pH 6.5 ranges from less than 5% denatured in one hour at 67.5°C. to greater than 95% destruction of the pigment at 85°C. in the same time period. Studies of the reaction kinetics show the over-all reaction to be greater than first order, thus apparently involving more than one molecule at a time in the denaturation process.

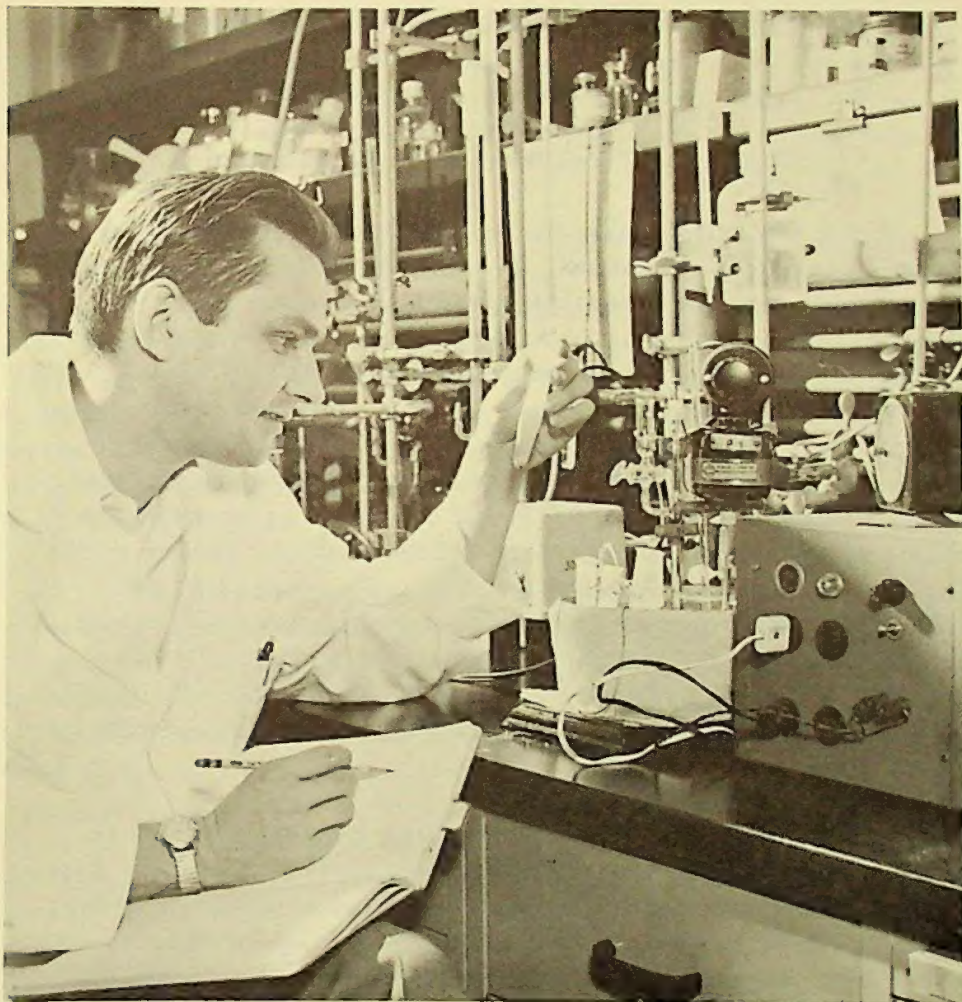
Assuming the initial rate of denaturation to be independent of the aforementioned effect and first order, rate

constants have been determined and the heat of denaturation found to be about 60,000 calories per mole. This value is similar to values found for other proteins, but the temperature range for denaturation is somewhat higher than normal. That the method of preparing myoglobin affects the physical characteristics is shown by the observation that the pigment prepared by ammonium sulfate fractionation is more heat stable than is myoglobin in crude extracts or myoglobin prepared by heat denaturation of contaminating proteins. The rate of heat denaturation varies with the pH, showing little change through the region of neutrality but increasing sharply below pH 6.0. At pH 5.4 denaturation is 90% complete in one and a half minutes at 75°C., while above pH 6.0 from 45-55% is denatured in two minutes at the same temperature.

An assessment of the effect of various salts, anions, and cations on the rate of denaturation of myoglobin has shown that the effects in general are non-specific. The addition of salts such as potassium chloride, ammonium sulfate, or sodium citrate tend to increase the amount of material denatured after a given period of time, but has no effect on the initial reaction rate. Thus, the primary effect of the salt appears to be on the denatured protein and not on the native material. This probably is due to the binding of anions and cations by ionized groups on the protein as the molecule is unfolded by thermal agitation.

The preparation of myoglobin by the thermal denaturation of extraneous protein has been tried and it is found that all proteins are removed except two unidentified components that make up some 30% of the remaining protein. These two components are larger molecules than myoglobin, as shown by their sedimentation behavior, but cannot be separated electrophoretically.

The studies discussed have all been carried out with metmyoglobin, and further studies using different oxidation states and complexes of the heme pigment are necessary to the understanding of the heating process.



A purified extract of cured-meat pigments is sought by A. J. Siedler in AMIF studies to determine the precise chemistry of meat-color pigments and of changes occurring during curing and other processes.

Chemistry of Production of Cured Meat Color

By A. J. Siedler

Division of Biochemistry and Nutrition

In order to study the variables associated with cured meat color fixation, a purified system was devised to approximate the conditions found in cured meat. Temperatures were maintained by immersing the tubes containing purified metmyoglobin (MetMb) and the other reactants in a water bath ($\pm 0.25^\circ\text{C}.$). All reactants were heated for 1 hour periods at the specific temperatures studied. The reaction mixture contained 3% ammonium sulfate (equivalent to 4.5% NaCl on an ionic basis) in order to approximate the salt concentrations in the actual curing procedure. Ascorbic acid and cysteine hydrochloride solutions were made up fresh prior to use and the pH of these solutions was adjusted to the pH of the reaction mix with NaOH. Sodium hydrosulfate (dithionite, $\text{Na}_2\text{S}_2\text{O}_4$) solutions were made up with H_2O flushed with N_2 just prior to use.

Heat precipitated MetMb was evaluated by acid hematin determinations of the precipitate. Reaction heme present after the various treatments was determined by conversion of the mixed water soluble MetMb derivatives to the nitrosomyoglobin (MbNO) derivative by the addition of excess NO_2^- and $\text{Na}_2\text{S}_2\text{O}_4$ crystals. The resulting spectrum obtained gives an estimation of the maximum amounts of water soluble reactive heme (capable of forming MbNO) present. The denatured MbNO (MbNO_2) -- the

"fixed" cured meat pigment) was determined by spectral analysis of the acetone extracts.

The total recoverable acid hematin + reactive heme of the supernatant solutions (as MbNO) + denatured MbNO↓ were converted to MetMb values. The difference between the total MetMb recovered and the original starting MetMb was estimated to be degraded or unreactive heme. The amount of degraded or unreactive heme is also classed as denaturation of MetMb.

The heat denaturation of MetMb under the conditions used in these studies shows that a relatively high temperature of 70°C. (158°F.) is needed to heat precipitate the major portion of MetMb at pH 5.5 in acetate buffer. Temperatures of at least 75°C. (167°F.) to 80°C. (176°F.) were needed to heat precipitate MetMb buffered in phosphate at higher pH's. The presence of NO₂⁻ at 50-fold excess over MetMb did not appreciably affect the heat denaturation results.

The presence of reducing agents has a significant effect on the denaturation of MetMb at 60°C. The addition of low level of Na₂S₂O₄ only slightly increased denaturation in the presence or absence of NO₂⁻. However, high levels of Na₂S₂O₄ caused 63-72% denaturation with little or no heme destruction.

The addition of 20 μmoles ascorbate caused 46-59% denaturation of MetMb. This denaturation could be completely accounted for as heme destroyed. Sodium nitrite protected the heme from attack by ascorbate. No additional denaturation other than heme destruction occurred with NO₂⁻ + ascorbate present.

Cysteine caused denaturation of 45-52% of the MetMb at 60°C. This denaturation also could be accounted for as

heme destruction. Spectral evidence indicates that at least part of this destruction is due to sulfmyoglobin formation. The addition of NO_2^- also protected the heme from destruction by cysteine.

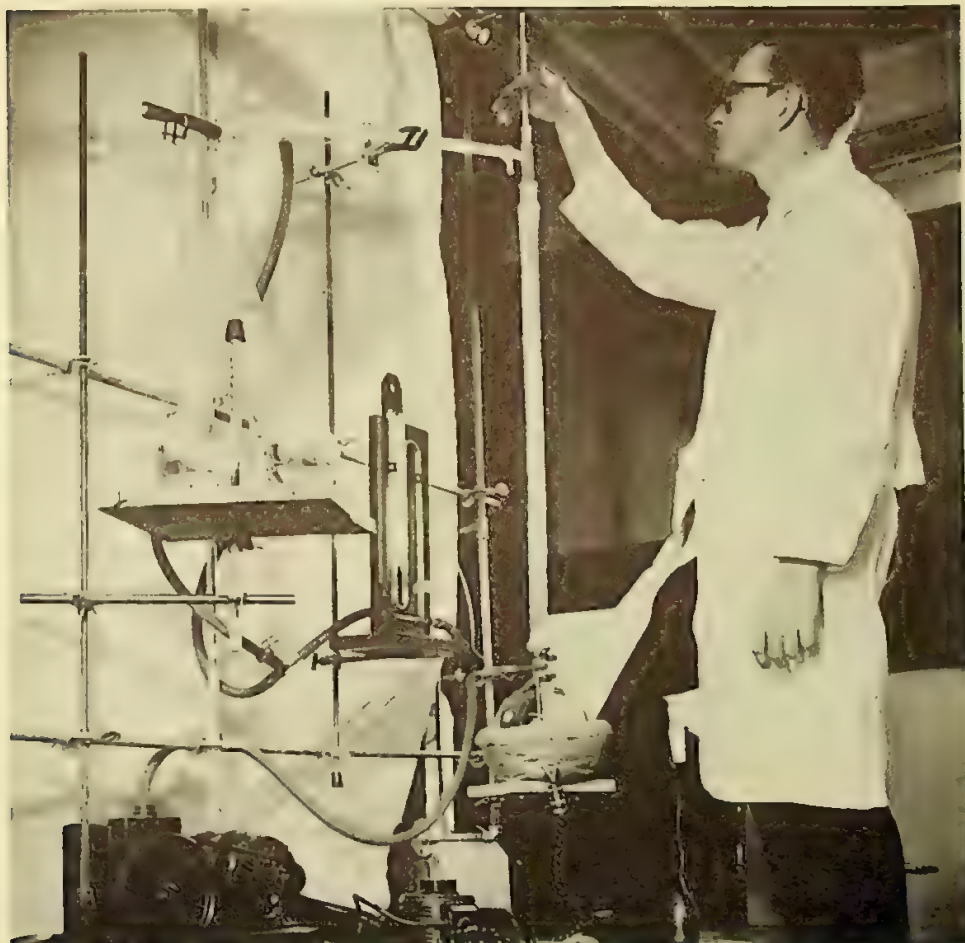
Comparisons of heme destruction at 60°C . and 70°C . in the presence of ascorbate or cysteine show that denaturation has some protective effect against heme damage by these agents. This protective effect is probably due to removal of the labile heme from the water soluble system during denaturation.

Only the systems containing high levels of $\text{Na}_2\text{S}_2\text{O}_4$, which caused appreciable denaturation of MetMb with the heme intact, were capable of forming the $\text{MbNO}\downarrow$ derivative at 60°C . whereas all the reductants tested were capable of forming $\text{MbNO}\downarrow$ at 70°C . The yield of $\text{MbNO}\downarrow$ in the system using $\text{Na}_2\text{S}_2\text{O}_4$ at 70°C . was lower than the yield of $\text{MbNO}\downarrow$ at 60°C ., probably due to the rapid exhaustion of $\text{Na}_2\text{S}_2\text{O}_4$ reducing activity at higher temperatures.

The reductants tested showed considerable variation in ability to form $\text{MbNO}\downarrow$ at 70°C . This variation was also dependent on the amounts of NO_2^- present in the system for each specific reductant used. The $\text{Na}_2\text{S}_2\text{O}_4$ system is apparently quite labile, and is the least efficient reductant for $\text{MbNO}\downarrow$ formation at 70°C . A high level of NO_2^- further decreases the efficiency of $\text{Na}_2\text{S}_2\text{O}_4$ at 70°C . Cysteine was of intermediary efficiency for $\text{MbNO}\downarrow$ formation at 70°C . with no significant variation in $\text{MbNO}\downarrow$ formation observed with high or low levels of NO_2^- . The yield of $\text{MbNO}\downarrow$ ranged from 40-50% with cysteine as reductant.

Ascorbate was the most NO_2^- sensitive reductant for formation of $\text{MbNO}\downarrow$ at 70°C . With low NO_2^- , a moderate yield of $\text{MbNO}\downarrow$ was obtained (22-40%) whereas, with high levels of NO_2^- , 62-72% yield was obtained.

These experiments indicate that the level of NO_2^- , the type of reductant present, and pH have a marked effect on the amount of cured meat pigment "fixed" by heat denaturation. Future experiments to further evaluate the effects of pH and the influence of other components, such as nitric oxide, have also been planned.



Hal T. Slover of AMIF Division of Organic Chemistry checks temperature of take-off during distillation of by-product residues of extreme fat-processing as he investigates technique for the preparation and measurement of fat-oxidation products.

Near-Infrared Spectra of Fat Oxidation Products

By Hal T. Slover

Division of Organic Chemistry

The present study deals with some of the spectral properties of oxidized fatty systems and of typical compounds found in such systems. The near infrared, in the region from about 700 to 3500 $\text{m}\mu$, has received more attention recently, since improved instrumentation has made this region more easily accessible, with greater resolution. Several papers have enumerated the spectral bands characteristic of this area. Some of these are of great interest to the fat chemist, particularly those in the 2700-3000 $\text{m}\mu$ range. Most of the oxygen-containing functional groups produced in autoxidized fats have characteristic absorption bands in this narrow segment of the spectrum. The fundamental -OH stretching absorptions of alcohols, hydroperoxides, and acids fall between 2750 and 2830 $\text{m}\mu$. The first overtone of the >C=O stretch of aldehydes, ketones, and esters is found near 2900 $\text{m}\mu$. It seemed that some of these bands might be helpful in the frequently tedious analysis of autoxidized fats and fatty esters.

The spectral curves were taken with a Beckman DK-2 Spectrophotometer on dilute CCl_4 solutions, using 1 cm. silica cells. Pure compounds were first studied to determine the precise location of the absorption bands. The free carboxyl -OH gives a very sharp, strong band at 2830 $\text{m}\mu$. The free hydroxyls of primary, secondary, and tertiary aliphatic alcohols fall very close together:

primary at 2750, secondary at 2755, and tertiary at 2760 μ . They could not be resolved in a mixture of the three. The hydroperoxyl of -OH of t-butyl hydroperoxide absorbs at 2808 μ ; that of cumene hydroperoxide at 2815 μ . The carbonyl overtone bands are much broader and weaker. Ester carbonyl absorbs at 2880 μ , aliphatic aldehyde at 2895-2900, and ketone at 2915-2920.

Alcohol and hydroperoxide bands are well resolved in mixtures. Solutions of impure hydroperoxide showed a well defined alcohol band. Hydroperoxide and carboxylic acid, however, mutually interfere and, in mixtures, give only one band. In a mixture of alcohol, hydroperoxide, and carboxylic acid, only two bands appear: an alcohol band and a combined band for hydroperoxide and carboxyl. All these -OH containing compounds are subject to hydrogen bonding, which lowers the absorption of the free hydroxyl and causes the appearance of dimer and polymer bands at higher wavelengths. The best resolution and the highest molar absorption are obtained on dilute solutions. The carbonyl bands are not resolved, although the position of the maximum will vary somewhat depending on the composition of the mixture.

Two samples of methyl oleate were autoxidized by bubbling O_2 through the heated ester and samples were taken at intervals for spectral curves. One was reacted at 35°C. to produce a high concentration of hydroperoxide substantially free of acid. In the spectra of successive samples, the absorption of the bands at 2815 and 2880 μ increased progressively. A plot of the absorption at 2815 vs. peroxide value gave a straight-line relationship at low acid concentrations. As the acid increased, the band maximum shifted toward 2830 μ and the relationship became non-linear. Only one carbonyl band was observed, increasing and shifting toward higher wavelengths, away from the ester frequency, as the autoxidation proceeded. Another

sample of methyl oleate was autoxidized at 100°C., producing a high concentration of acid. The acid-hydroperoxide and carbonyl bands again increased, and a substantial alcohol band appeared. The 2815 mμ maximum shifted toward the acid absorption frequency and its magnitude was not linear with peroxide value.

To further compare the effect of known variations in composition on absorption, portions of a sample of autoxidized methyl oleate high in hydroperoxide and acid were (1) washed with mild base to remove acid, (2) washed with KI to reduce peroxide, and (3) subjected to both treatments. Removal of acid decreased the acid-hydroperoxide absorption band and shifted it toward the lower hydroperoxide frequency. Similarly, hydroperoxide reduction also decreased this band and shifted its maximum toward the acid absorption frequency. At the same time, the hydroxyl formed by the reduction could be detected by the increase in the hydroxyl band. When both acid and hydroperoxide were decreased, the band at 2815-2830 mμ was correspondingly lowered. In each treated sample the carbonyl absorption was lowered, perhaps due to loss of water-soluble aldehyde.

As shown by these few experiments, the near infrared can be most useful in the examination of fatty autoxidation products. Quantitative applications, however, are complicated by band interference and hydrogen bonding. Further work in this field may suggest methods of coping with these problems.

Preliminary Studies on Rancidity in Pork Products

By L. R. Dugan

Division of Organic Chemistry

The failure of both untreated and cured pork to maintain palatability and freedom from rancidity during storage at low temperatures or in the frozen state has imposed a great stress on the economic production, handling, and distribution of pork items. A better understanding of the nature of rancidity in pork products is necessary to provide a basis for solution of some of the problems raised.

It was deemed desirable to initiate studies in pork rancidity on a very fundamental basis and to attempt to develop knowledge which will reveal the nature of rancidity in meat products and how it differs from rancidity in a fat that has been separated from the complexities of the whole meat system.

It is probable that, in the complex tissue system, the polyunsaturated fatty acids in the fats are the sites of initial oxidative rancidity. This would compare to the separated fat system, but there the similarity may end. Further information is needed on the possible influence of components of the system such as the water, salt, curing salts, proteins, enzymes, heme compounds, oxygen solubility, and other factors on the initiation and maintenance of oxidation reactions which lead to rancidity.

It was decided to study the problem first through model systems and then to develop more complex systems. Linoleic acid, the major polyunsaturated fatty acid in pork, is present in the fat of the average corn-fed hog at a level of approximately 10%. The oxidation of linoleic acid and its esters in the pure state is fairly well understood. Linoleic acid and methyl linoleate were, therefore, selected as the model compounds of choice for the initial studies.

Studies have been made of the oxidation of linoleic acid and methyl linoleate in aqueous buffer solutions. The buffers control pH and the aqueous system imposes conditions more nearly like those found in whole tissue. Oxidation was measured as a function of oxygen uptake of the suspensions in a Warburg apparatus. The suspensions were prepared at concentrations of 0.1 molar by sonaration with sonic waves of 10 kc. Graphical treatment of oxygen uptake data gives straight lines corresponding to:

$$\frac{dO_2}{dt} = K \triangleleft O_2 = K \cdot ROOH.$$

The reaction constants for the oxidation of linoleic acid at four temperatures are shown in this table.

<u>Temperature</u> °C.	<u>Reaction Constant</u> K Min ⁻¹
20	1.17 x 10 ⁻³
25	1.89 x 10 ⁻³
35	4.53 x 10 ⁻³
40	8.10 x 10 ⁻³

The activation energy of linoleic acid suspension at pH 7 oxidized under these conditions is calculated to be E = 16,300 cal./mole. A similar treatment for methyl

linoleate provides $E = 17,600$ cal./mole. These compare with literature values for an energy of activation for oxidation of methyl linoleate in the pure state at $18,000$ cal./mole. Treatment of the data from numerous experiments by kinetics allows an evaluation of the mechanism of reaction. It is necessary first to initiate oxidation, and the over-all reaction may be depicted as:



This undoubtedly requires a large energy of activation. Further reaction depends greatly on peroxide decomposition reactions. The first appears to be monomolecular: $ROOH \rightarrow RO\cdot + \cdot OH$ at low hydroperoxide concentrations, and this tends to give way to a bimolecular reaction: $ROOH + ROOH \rightarrow RO_2\cdot + RO\cdot + H_2O$. The monomolecular decomposition has never been well observed in studies with pure linoleates. This stage can be shown readily in the aqueous suspension. The bimolecular decomposition is the most readily observed and this leads to the attainment of a steady state reaction from which the limiting rates for oxygen uptake may be determined.

Attainment of a homogeneous solution of linoleate ester in water is impossible but the use of linoleic acid with a NH_3/NH_4 buffer to maintain a pH of 9 gave some interesting results. The kinetics of the oxidation reaction with this system were like those of a heterogeneous system. Only when the pH was raised to 10 was there evidence for reaction as expected in a homogeneous system. The reasons for deviation at pH 9 are obscure but appear to be related to formation of a colloidal system of aggregates of micelles.

Meat systems have a pH of 5.8-6.0 and this has directed attention to investigation at pH values on the acid side. Emulsion stability is a greater problem here.

Results are not conclusive but they do not confirm literature reports which indicate that pH in the interval 5.1-9.2 has no influence. The lower pH systems seem to have affected the course of the oxidation. It may be that the pH of the system influences the hydroperoxide decomposition reactions to determine whether they will be mono- or bimolecular. Our results indicate that lower pH may direct toward a monomolecular decomposition.

There are many factors to be evaluated but this approach through reaction kinetics appears to offer promise for understanding the causes of rancidity in pork products.

Problems Encountered in the Detection of Antioxidants in Fats

By Nancy Risser

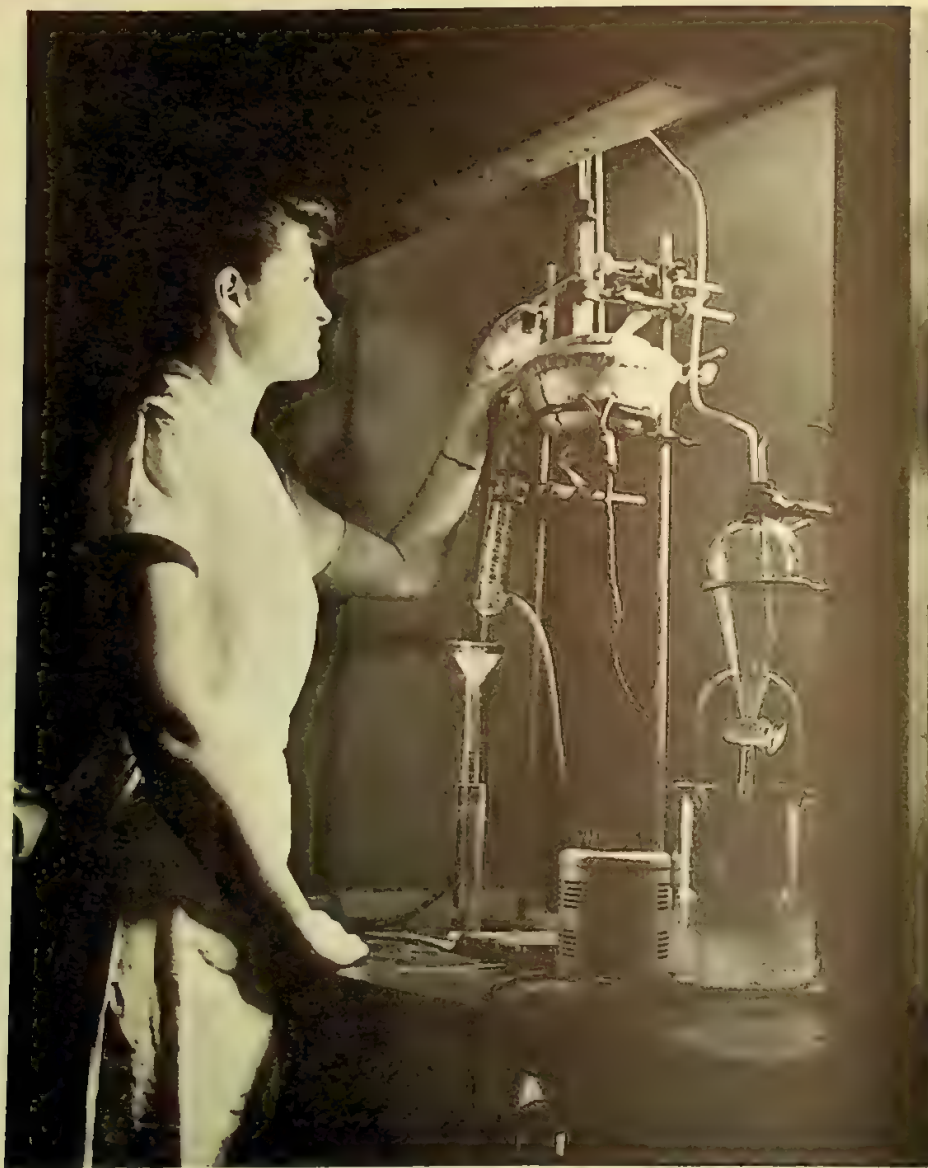
Division of Organic Chemistry

The development of antioxidants for lard proved to be a major factor in the increased acceptance of animal fats for shortening and commercial baking. The major portion of the great quantities of treated lard was stabilized under Federal inspection, and labeling requirements indicated the maximum quantities of each antioxidant that could be used. Large scale users of antioxidants tended to use less than the maximum amounts permitted by regulation after learning that lesser quantities would usually provide required stability values.

The development of methods for qualitative and quantitative determination of the antioxidants, long a matter of concern of regulatory officials, has been given added emphasis by the rejection of substantial quantities of United States lard imported into Germany where no antioxidants are permitted.

The antioxidants of greatest concern are propyl gallate (P.G.), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and nordihydroguaiaretic acid (N.D.G.A.).

A recent method, developed by Mahon and Chapman, makes possible the determination of these antioxidants singly and in combination except when N.D.G.A. and P.G.



Nancy Risser of Organic Chemistry Division runs one of series of divers experiments in efforts to develop rapid and efficient qualitative and quantitative analyses for antioxidants in fats.

may be used together.

Extraction methods frequently require time consuming and laborious techniques, as well as impose serious limitations on the quantitative recovery of some antioxidants from lard.

The extraction of N.D.G.A. and propyl gallate involves dissolving the fat in CCl_4 and extracting the antioxidant with 50% ethanol. The antioxidants in the extracts are determined colorimetrically from the colors developed by treatment with ferrous sulfate in buffered systems. BHA and BHT which are partially extracted by the procedure do not interfere with this colorimetric method.

Because of the time factors and poor extraction efficiency encountered in this method, we developed a procedure which combines simplicity and better extraction efficiency for propyl gallate. The antioxidant was extracted by heating the lard sample to 90°C . in a beaker in a boiling water bath. Thirty ml. of 50% ethanol were added and the mixture was vigorously stirred with an electrically driven wire stirrer for 2 minutes. The beaker was placed in a refrigerator until the lard layer solidified. The alcoholic layer was poured off into a volumetric flask and the procedure repeated. A third similar extraction with distilled water completed the extraction. The improved efficiency of the method compared with that of the previously used method is shown in the table.

Extraction of Propyl Gallate from Lard

Concentration of Propyl Gallate in Fat	Efficiency of Recovery	
	Mahon-Chapman	New Method
.005%	40%	95%
.01%	--	97%
.02%	35%	70% Hand 95% Mechanical

This method with 50% ethanol has been only about 75% effective in extracting N. D. G. A. Use of higher alcohol concentrations may provide efficiency equal to that encountered in extraction and determination of propyl gallate.

Extraction of BHA can be accomplished quite well by use of 80% ethanol but this method extracts only about 25% of the BHT in a fat. Application of the steam distillation method of Mahon and Chapman appears to show promise for quantitative estimation of both these antioxidants. When both antioxidants are present, the sum of BHA and BHT is determined by the ferric chloride γ , γ -dipyridyl reaction. BHA is determined alone by a color reaction with 2,6-dichloroquinonechloroimide. BHT is then determined by difference. In these determinations, it is imperative that the reagents used be fresh and the solutions of the color-forming reagents should be prepared daily for best results.

The Germans used a spot test to determine the presence of gallates in lard. This consisted of stirring a small quantity of melted lard with NH_4OH . A pink color in the lard, which faded in a few minutes, indicated gallates. The method can be confusing at low levels of gallate. A method involving a single extraction with 50%

ethanol, as used in the quantitative method cited above, provides a more sensitive test. A pink color, which appears and fades away with addition of 1 ml. NH_4OH to 5 ml. of the extracts, is a sensitive test for gallates.

Currently available methods of antioxidant analysis give reasonably satisfactory results. The solution of many problems, of which those mentioned here are only a fraction, is necessary to expedite time saving and provide better precision and confidence in the methods.



The shortening is extracted from frozen pastry by Audrey Freeman of Home Economics as a step in measuring the oxidative products of fat in freezer stored pastry.

The Stability of Pastry Doughs Containing Animal Fats Treated with Antioxidants

By Audrey Freeman

Division of Home Economics

The use of frozen pre-cooked foods has expanded rapidly in the past years. Information on the stability of fats and the effectiveness of antioxidants in frozen foods is limited. The presence of antioxidants in frozen pre-cooked turkey products was reported to retard the development of peroxides and of rancid flavors [Lineweaver, H., J. D. Anderson, and H. L. Hanson. Effect of antioxidant on rancidity development in frozen creamed turkey. *Food Technol.*, 6, 1-4 (1952)], and naturally occurring oxidation-retarding materials such as found in soy flours have been shown to inhibit peroxide formation in frozen raw and cooked pastry [Overman, A. Antioxidant effect of soybean flour in frozen pastry. *Food Research*, 12, 365-371 (1947)]. The efficiency of the commonly used chemical antioxidants in protecting fats from oxidation at low temperatures merited further investigation.

Data on pastry doughs prepared with prime steam lard, lard stabilized with two commercial antioxidant preparations, and a hydrogenated vegetable shortening are presented here. The antioxidant preparations tested were: Tenox II containing BHA (butylated hydroxyanisole), propyl gallate, and citric acid; and Tenox IV containing BHA and BHT (butylated hydroxytoluene). Dough samples were heat sealed in an oxygen impermeable cellophane and stored at -10°F. for up to 36 weeks. The doughs were allowed to thaw at room temperature before opening the wraps.

A small portion of each dough was removed for free fatty acid and peroxide analyses. The remainder was baked and distributed among five jars for accelerated storage at 145°F. in a Schaal oven.

Fresh pastries containing unstabilized lard had a mean Schaal oven life of 17 days; pastries from similar doughs stored at -10°F. were as stable. The fat extracted from the unstored doughs had a peroxide value of 3.4; after two weeks of freezer storage the peroxide value had increased to 5.4. Longer storage periods did not increase the amount of peroxide present. The highest free fatty acid value in the unstabilized lard samples also was found in doughs stored two weeks. The acid value was less in doughs stored for longer periods.

The doughs containing lard stabilized with Tenox II had the same Schaal oven life after two weeks of storage as the fresh doughs. However, further storage decreased the stability of the pastries. Fat extracted from freshly prepared dough had a peroxide value of 2.4; the peroxide value was higher (2.8 to 6.6) in stored doughs. The free fatty acid values reflected the same trend as did the Schaal oven life. This finding is discussed in more detail later in the report.

The mean stability of fresh pastries stabilized with Tenox IV was 108 days. Pastries from the stored doughs were less stable than the fresh samples except for those stored 20 weeks. The fat extracted from fresh dough had a peroxide value of 2.0. The peroxide value of stored doughs ranged from 4 to 6 except in the 12 week sample which had a peroxide value of 15. As in the doughs containing Tenox II, the free fatty acid values showed the same trend as the Schaal oven life.

Doughs made with hydrogenated soybean oil were

more stable after freezing and storage. They had an initial Schaal oven stability of 40 days; after 8 weeks of storage the mean stability was 98 days, and after 20 weeks it was 88 days. The peroxide value of the fat extracted from the unstored dough was 9.8. All stored samples had a lower peroxide content. The initial free fatty acid value was 0.21 and increased to 0.44 or higher in all the stored samples.

The stability of the pastries varied throughout the storage period. The variation in pastries made from prime steam lard was generally within the limits which could be due to the Schaal oven method of measuring stability. Similar trends were shown by the stabilized fats, but the differences were more pronounced and definite peaks representing increased stability of the fats appeared at 20 weeks. Peaks were observed, also, earlier in the storage period, occurring at 6 and 10 weeks with lard and Tenox II, at 8 weeks with lard and Tenox IV, and at 4 and 8 weeks with hydrogenated shortening. Actually, frozen doughs made with stabilized fats can be stored for 6 months or more without becoming rancid.

The peroxide and free fatty acid values reflected this change in Schaal oven stability. The peroxide values varied inversely with the Schaal oven life during the beginning of the storage period. Changes in free fatty acid content would be expected to concur with changes in peroxide value and to be lower in the more stable pastry samples. Actually, an increase in free fatty acid content coincided with an increase in pastry stability.

This unexpected increase in free fatty acids may be the result of an accumulation of free fatty acids from the hydrolytic action of lipases present in the flour, or it may suggest a step-wise oxidation of the fat. The latter could be explained by oxidation and breakdown to

free fatty acids in the complex system and then a further storage period before second stage oxidation occurred. Observations on fat oxidation and antioxidant systems have been made generally on fat alone, and the changes may not necessarily appear in the same way in pastry.

The stability of fats in frozen doughs has been studied using Schaal oven storage and peroxide and free fatty acid measurements. Fat stability fluctuated throughout 6 months of storage, but pastries baked from doughs made with stabilized fats did not become rancid during this period.

GENERAL

Nutritional Studies on the Relation of Fat and Protein to Atherosclerosis

By C. H. Lushbough

Division of Biochemistry and Nutrition

During the past several years, there has been increasing research interest in atherosclerosis, the major type of heart disease, and the leading cause of mortality in the United States at the present time. Atherosclerosis is a degenerative disease in which the arteries, including the aorta and the coronary arteries, become progressively infiltrated with the deposition of fatty materials beneath the internal lining of the vessel. This process leads to a thickening and reduction in elasticity of the artery, with reduction in the blood-carrying capacity of the blood vessel involved; and eventual occlusion of the vessel or formation of a blood clot (thrombus) at the site of the atheromatous lesion may occur.

Many different factors have been implicated in atherosclerosis, including sex and hormone balance, hereditary predisposition, stress factors, and nutrition. A research project has recently been undertaken in the laboratories of the American Meat Institute Foundation to evaluate the nutritional interrelationships between the type and amount of fat and protein in the diet and the development of atherosclerosis in the male albino rat.

A series of experimental rations was designed to provide low and high levels of lard or corn oil in combination with graded levels of casein. The fat tested was

substituted isocalorically for sucrose at the high fat levels, and minerals and vitamins were provided in a constant ratio to total calories in all rations. Male weanling rats were fed ad libitum until they achieved body weights of 400 g., when restricted feed intakes were employed to maintain constant body weights in the range of 400 g. \pm 15 g., for the remainder of the experiment. After six months, tail vein bleeding provided blood serum samples from each animal for further analysis. The serum contents of cholesterol and, in certain cases, the polyunsaturated fatty acid contents of the serum were determined. Similar determinations are also to be undertaken for the 9- and 12-month periods and, at the end of the 1-year experiment, the hearts and aortas of the experimental animals will be examined for evidence of the development of atherosclerotic lesions.

The growth response of the young rats fed experimental rations containing high levels of fat confirmed the results reported from the Foundation laboratories in earlier studies with the dog and the chick. At both low and high levels of casein in the ration, the substitution of 15 parts of lard for an isocaloric amount of sucrose resulted in increased growth rates and increased efficiency of feed utilization. Thus, for example, with the 12% casein ration, the isocaloric substitution of the increased amounts of lard resulted in 5.7 g. gained/100 Cal. gross food intake, compared to 5.5 g. gain/100 Cal., at the 5% lard level during an 8-week test period. With 36% casein, the effect was more pronounced, and values of 7.7 g. gain/100 Cal. were observed at the high fat level, compared to 7.2 at the 5% level of lard. Similar effects were noted with corn oil.

At high levels of fat in the diet, both with and without 1% cholesterol added to the diet, the serum cholesterol values observed for corn oil were generally somewhat lower

than those observed when lard was fed. The addition of 1% cholesterol to the high-fat diets resulted in significant increases in the serum cholesterol values in every group. The effect of increasing levels of casein on the level of serum cholesterol was not clear-cut, and the evidence obtained from the 6-month results indicates that the level of serum cholesterol in the young adult rat is relatively unaffected by the amount of protein in the ration.

Preliminary studies on the polyunsaturated fatty acid content of the serum of two to four animals from each of several of the experimental groups suggest that at 6 months the serum level of fatty acids with two double bonds (linoleic acid) may be significantly affected by dietary protein levels and also by the type and amount of fat in the diet. Serum diene (linoleic acid) values decreased progressively with increasing levels of protein in the ration when either lard or corn oil was fed, and generally lower levels were observed at the lower fat intake levels. The effect of increasing levels of protein, and of low and high levels of lard or corn oil, on the other polyunsaturated fatty acid fractions studied (trienes, tetraenes, pentaenes, and hexaenes) was not clear-cut.

Although further work is required to complete these studies on a larger number of animals included in this experiment, it would appear that the relation between the type and amount of fat and protein in the diet and the development of atherosclerosis may be more closely related to the metabolism of the polyunsaturated fatty acids than to cholesterol metabolism per se.

Further studies of the blood lipides of the animals included in this experiment after 9 and 12 months and examination of the hearts and aortas may be expected to provide further significant information on the fundamental relationship between the kind and quantity of fat and protein in the diet and the development of atherosclerotic heart disease.

Energy Content of Animal By-Product Feeds

By Marion Cullen

Division of Animal Feeds

During the past six years, the study of energy nutrition of poultry and animal rations has been stimulated by the discovery of the presently acknowledged value of fat in feeds for live stock and poultry. The determination of the energy value of feed ingredients is important in providing a more exacting guide in formulating high energy, high efficiency feeds. In keeping with the present trend indicating a continued ready supply of feed grade fats, we have undertaken the determination of the energy content of the various animal by-products commonly used in feeds.

In undertaking the study of energy nutrition, it is paramount to recognize that the fundamental problem is to provide sufficient fuel for survival. The amount of B vitamins or specific minerals or any particular nutrient is influential, but is not the principal consideration. Energy values of feeds are expressed in Calorie units because the energy content of a diet consumed can be measured in terms of heat produced. To evaluate the energy content of feedstuffs by indirect calorimetry, the gross or total energy of the sample is first determined in a bomb calorimeter. The caloric content thus derived represents the potential energy value of the ration. Unfortunately, the gross energy of the ingested diet is not always completely utilizable



Data on the gross energy content of animal fats, measured with an oxygen-bomb calorimeter, is used by Marion Cullen of AMIF Animal Feeds as basis for determination of utilizable energy in chick rations.

and, therefore, not always equivalent to the number of Calories the animal can obtain from the feed. This discrepancy arises for several reasons: (1) part of the diet may be undigestible, (2) the protein fraction of the diet is not so completely oxidized in the animal body as it is in the calorimeter, and (3) a portion of various nutrients in the diet may be stored in the body tissues. Therefore, it is evident that the gross energy content of any ration does not provide all of the information needed.

In an effort to evaluate a ration according to how well it supplies an animal with the energy needed for physiological functions, it is necessary to account for various losses which occur. It seems most reasonable to measure the caloric content of the fecal loss or indigestible residue. Then, by subtracting the caloric content of the diet lost as feces from the total gross caloric content of a known amount of feed, we have the remainder as the energy which is available to the animal body through absorption and digestion. The energy value derived by this means is known as digestible energy. Knowing the digestible energy content of feed, one could then formulate rations on the basis of how much energy is removed from the feed by an animal as the material passes through the gastro-intestinal system.

Digestible energy is relatively easy to determine, but with birds it is difficult to arrive at true digestible energy values because the undigestible residues and urinary wastes are excreted together. Consequently, when measuring the energy value of poultry rations, it is more accurate to determine metabolizable energy. To account for the protein fraction not completely oxidized and as such excreted as uric acid, there are corrections made in computing the final metabolizable energy content of the diet. Metabolizable energy represents the energy of the feed consumed which is available to the

animal for body maintenance plus growth, egg production, milk production, etc. Metabolizable energy is not so susceptible to varying conditions. It is an index of the usable energy in the ration because metabolizable energy is a measure of all that is absorbed and that can be used for whatever purpose is paramount.

In the past few years research workers have been accumulating data on the metabolizable energy content of feedstuffs. Using such information as a guide, more accurate standards for computing economical, practicable diets can be developed.

Our work has thus far involved the determination of the metabolizable energy content of feed grade fats. We are using the method developed by the poultry husbandry department of Cornell University, with a few modifications. To date we have metabolizable energy values for five different samples: Choice White grease, 4086 Calories per pound; corn oil, 4129 Calories per pound; and several other fats currently being offered to the feed trade, which range from 3028 to 3666 Calories per pound. From the data obtained on the energy content of the samples, it can be seen that the energy in Choice White grease and corn oil are more available to the chick.

Our value for Choice White grease is quite comparable to the value the Cornell group reported for lard (3960 vs. 4086). Also, we obtained a very similar value for corn oil (4070 vs. 4129), indicating that the method is reproducible within reasonable experimental error. We are continuing to study the metabolizable energy content of animal by-product feeds for use in high energy broiler rations. At present, our work is concerned with determining the metabolizable energy content of animal fats of different types and grades to see if composition, titer, or grade might influence the energy value.



Dr. J. Walter Giffie, Chief of the Division of Hide Research, utilizes Foundation's electrophoresis equipment in study of composition and structure of hide collagen and related proteins.

Characterization of Hide Components

By J. W. Giffie

Division of Hide Research

The Division of Hide Research is concerned principally with the major component of hide, collagen. The importance of this protein is not, however, confined to the skin of the animal for, taking into account bone and connective tissue, collagen comprises 30% of the mammalian body protein. Therefore, basic findings regarding composition and structure of collagen and related proteins find a broad scope of application, especially in uses for hides and skins.

In recent years, much knowledge has been added on the chemistry of collagen, and its complex structure has gradually yielded to a variety of investigative approaches. One aspect, however -- the relationship of carbohydrate to the structure -- remains obscure. There have been several reliable reports which suggest that glucose, galactose, and glucosamine are intimately related to the collagen molecule. The manner in which the carbohydrate is associated with the protein is not indicated in any of these studies. What is apparently required to determine this association is careful purification of collagen material and mild degradation followed by isolation of the carbohydrate-rich fragments. A repeat of this pattern of approach upon the carbohydrate-containing fragments should reveal the site of carbohydrate linkage provided it is covalent in nature.

One of the obstacles to an approach such as that described above is the lack of adequate purity standards for collagen materials. Examination of the amino acid composition of collagen reveals that collagen contains approximately 14% hydroxyproline while tyrosine is present on the order of 1%. On the other hand, most of the soluble proteins of tissue contain appreciably greater concentrations of tyrosine and no hydroxyproline. It follows that, as the soluble proteins of a collagen-rich tissue are extracted, the ratio of hydroxyproline to tyrosine will rise to a relatively constant value.

It is obvious that, in order for such a ratio to be meaningful, the method of measurement of tyrosine must be accurate and precise. A spectrophotometric method for measuring tyrosine in hydrolysates has been developed with accuracy and precision of approximately 1%. The method takes advantage of the shift in peak absorbency to 293-295 millimicrons at a pH greater than 13. Studies with possible interfering substances revealed that the absorbency of chondroitin sulfate measured in the range 288-310 m μ increases 10-fold after acid hydrolysis. It was found, however, that a standard correction factor could be derived from hydrolysates of chondroitin sulfate by measuring the absorbency at 293 m μ , which is the tyrosine maximum, and at 345 m μ where tyrosine does not absorb. The ratio of these values is approximately 1.8 and, when multiplied by the absorption of crude collagen hydrolysates at 345 m μ , gives a correction factor which is subtracted from the 293 m μ absorbency. This approach has been evaluated and yields theoretical values for tyrosine.

For the estimation of hydroxyproline, the method of Troll and Cannan is used. It is attractive because of its simplicity and desirable sensitivity. The method is based upon the ninhydrin reaction which takes place in an

aqueous medium and is simultaneously extracted with benzene. The dye, although unstable in aqueous solution, is completely extracted by the benzene where it is relatively stable and exhibits an absorption maximum at 570 m μ . We have had considerable difficulty in achieving satisfactory precision with this method, but it now appears to be on the order of 2 to 3%.

For purposes of exploration, some of the extraction techniques reported by other workers in this field have been applied to thin layers of hide corium obtained by use of a meat slicer. Each reagent indicated below was employed repeatedly until the nitrogen analysis fell near zero. The residue was then extracted with the next solvent, and so on.

- (1) 10% NaCl
- (2) pH 9.3 Phosphate
- (3) pH 3.9 Acetate
- (4) 10% (0.775M) CaCl₂

Sodium chloride in solid form was added to the acetate extracts to provide a concentration of 5% salt. The protein precipitate so formed was suspended in water and dialyzed to remove the acetate and NaCl. A number of the fractions obtained by these procedures were subjected to tyrosine analysis. For descriptive comparison, X is designated as the amount of tyrosine per unit weight.

NaCl extraction residue	4X
Phosphate extraction residue	4X
Acetate extraction residue	3.5X
Acetate-NaCl precipitate	X
CaCl ₂ extraction residue	X

The last figure is of particular interest since this reagent is normally used to remove mucopolysaccharide.

Yet, it apparently removed a tyrosine-rich component, and is similar in this respect to the action of strongly basic extractants with which some hydrolysis is anticipated.

With methods for following the purification of collagen from hides, it should now be possible to examine the nature of carbohydrate present in the purified collagen.



Robert H. Deibel of the Division of Bacteriology prepares semi-synthetic medium utilized in studies to determine nutritional conditions that foster or retard growth of microorganisms sometimes found in canned hams and other meat products.

**Lipoic Acid Requirement for Anaerobic Utilization
of Pyruvate as an Energy Source
by *Streptococcus faecalis***

By Robert H. Deibel

Division of Bacteriology

Enterococci are known to be capable of using a wide variety of carbohydrates, polyalcohols, and organic acids as an energy source. Pyruvic acid is known to be an intermediate in many of these fermentations and is known to be oxidized by some enterococci. However, in the past, pyruvate has not been considered to be a suitable energy source for anaerobic growth of enterococci. The present study has shown that *Streptococcus faecalis* and its varieties characteristically ferment pyruvate in a tryptone, yeast-extract medium achieving a level of growth that equals or surpasses that obtained with glucose. On the other hand, *S. faecium* and *S. durans* cannot ferment pyruvate under these conditions.

When grown in a semi-synthetic, casein-hydrolysate medium with pyruvate as the energy source, *S. faecalis* has a nutritional requirement for lipoic acid. However, lipoic acid is not required in such a medium with various hexoses, pentoses, hexitols, or glycerol as the energy source. When citrate is supplied as the energy source, lipoic acid is again required, thus suggesting that the metabolism of this compound is related to the metabolism of pyruvate.

The growth of *S. faecalis* in the tryptone, yeast-extract medium proceeds rapidly without lag. However,

growth in the semi-synthetic medium often occurs after a 24-48 hour lag period. The irregular nature of the growth response in this medium suggested an adaptation to the utilization of this compound. A prompt growth response in the semi-synthetic medium could be obtained if the inoculum culture was adapted to pyruvate.

Lipoic acid previously has been shown to function in the oxidation of various alpha keto acids, including pyruvate. In addition, various microorganisms can be cultured in such a manner that lipoic acid is stimulatory or mandatory. The work reported herein presents evidence of the essential role of this growth factor in the anaerobic metabolism of pyruvate by growing cultures of S. faecalis. It also indicates the presence of a new pathway of energy metabolism in this organism.

The Etiology of Pork Tongue Abscesses

By James N. Campbell

Division of Bacteriology

Recently our attention was called to the fact that a rather widespread occurrence of pork tongue abscesses was being encountered by a number of meat industry companies. These abscesses had a reported incidence of some 3 to 6% of the animals slaughtered. However, since it is necessary to cut the tongue open for inspection for this condition, healthy as well as abscessed tongues become somewhat mutilated, thus restricting their use by the meat industry.

These abscesses, ranging in size from several millimeters to 1 centimeter, are found in the muscle at the basal area of the tongue. The location and size indicated that these abscesses were different from pork abscesses heretofore described in the literature.

Samples for this laboratory were obtained from a local packing house. As a result of the gross examination of some 85 abscesses, the following classification scheme evolved:

Young abscesses - connective tissue capsule
enclosing a small amount of purulent material.

Intermediate abscesses - connective tissue capsule
is more clearly defined and contains a large
amount of creamy purulent material.



Working in close collaboration with AMIF Division of Histology, James N. Campbell of Division of Bacteriology conducted study which identified straw-like foreign body as causative agent in certain types of pork tongue abscesses. Foreign particles were found to be part of a grass, probably from feed.

Old abscesses - connective tissue capsule is clearly defined. Contents of abscesses are less creamy and more granular. The purulent material may have a greenish cast.

The contents of 14 abscesses were examined for aerobic and anaerobic bacteria and preliminary attempts were made to identify the isolates. The bacteriological picture varied from abscess to abscess, ranging from one which appeared to be sterile to those containing several kinds of bacteria. This lack of a consistent bacteriological picture would not allow the implication of a bacterial species as the causal agent.

In one of the first young abscesses examined, a straw-like foreign body, 3 to 5 mm. in length, was found. Subsequently, careful examination of all abscess contents was made, and the same type of foreign body was demonstrable in nearly 75% of those examined. Similar foreign particles were found in all three types of abscesses. Those found in the older forms, although easily overlooked in the more abundant purulent material, gave evidence of having been partially broken down.

The foreign bodies were examined grossly and microscopically by Dr. C. E. Olmstead and Dr. B. F. Palser, of the Department of Botany, The University of Chicago. Histological sections were prepared by Marion Birkner and Keith Huber, of the Division of Histology, at the Foundation. As a result of these examinations, it was concluded that the foreign particles comprised a part of a grass, either part of an awn or, more probably, part of the small stem supporting the flower. Since the structures observed are characteristic of a large number of grasses, further identification of the samples supplied could not be made.

It appears, then, that the etiological agent for the

abscesses examined is a small, sharp part of a grass, perhaps representing one species of plant, which penetrates the tongue of the animal. Sharp spines, or epidermal hairs on the surface of the foreign body, prevent its withdrawal and it is worked in deeper by muscular action of the tongue.

Whether these foreign particles originate from some feed constituent or from pasture feeding is not known at this time. They appear to enter the tongue at an early age of the animal. It would be of interest to determine the geographic areas from which these affected hogs originate.

PROCESSING AND PRESERVATION

Further Studies on Chemical Changes that Occur in Beef During Irradiation

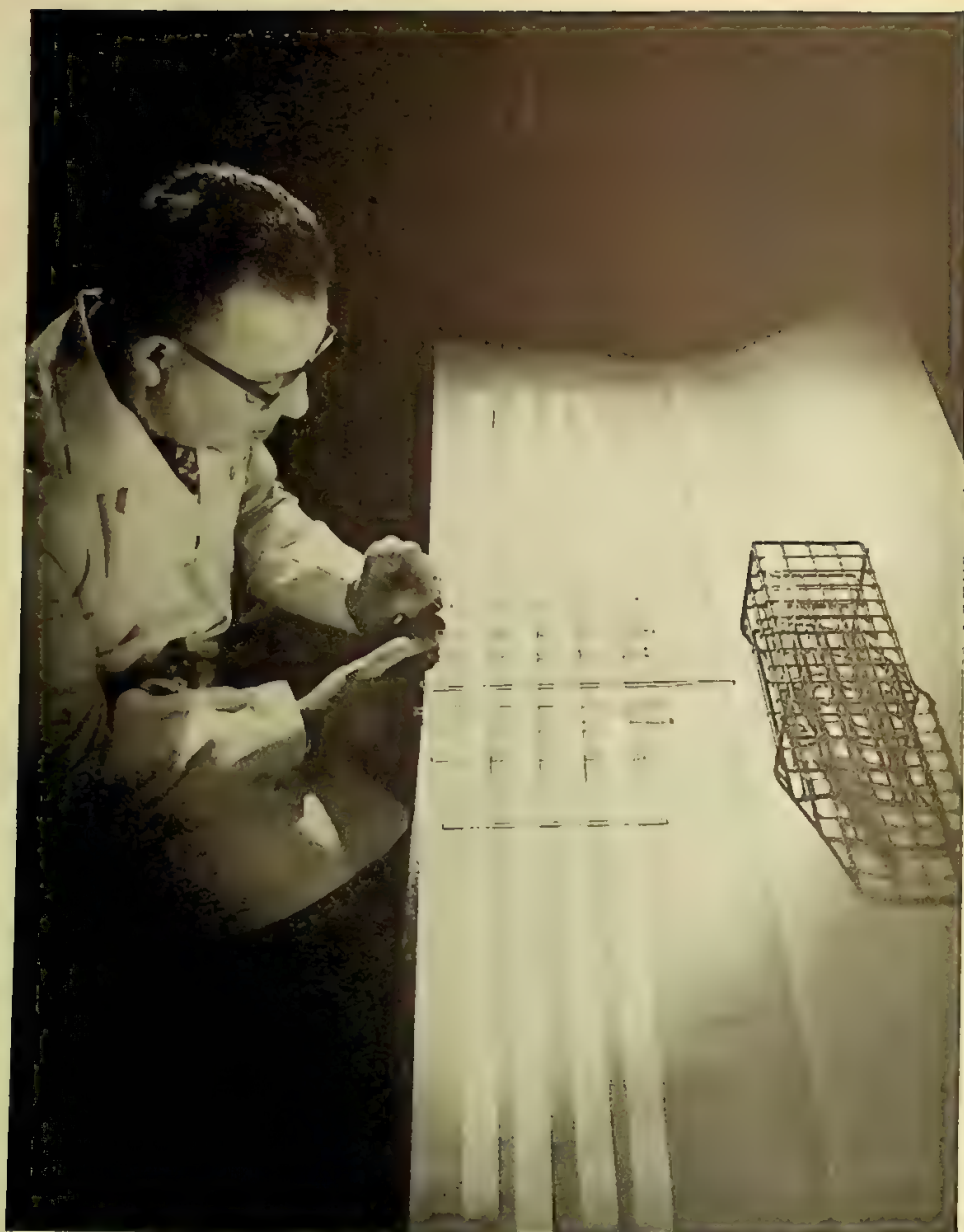
By O. F. Batzer

Division of Analytical and Physical Chemistry

The possible application of beta or gamma irradiation for sterilization of fresh meat and meat products has been under investigation by a number of laboratories both here and abroad. Although this process does yield a sterile product which can be kept for long periods without refrigeration, undesirable changes occur during irradiation which result in off-odors, off-flavors, and color changes in certain meat products, particularly beef.

Previous research at the Foundation led to isolation and identification of some of the compounds responsible for off-odors. Quantitative methods were developed or adapted to follow some of the chemical changes. Among these were increases in hydrogen sulfide, methyl mercaptan, acid-soluble carbonyl compounds, and pH. The disappearance of glutathione and glycogen was also followed. The results obtained from these experiments indicated that certain variables such as sample difference, age, and temperature affected the degree of chemical changes that occurred during irradiation. By controlling some of these variables it might be possible to gain some insight into the mechanisms involved in off-odor production.

An experiment was set up to determine what effect pre- and post-irradiation storage time and temperature,



Paper chromatography is utilized by O. F. Batzer for identification of compounds and derivatives of irradiated meat.

radiation dosage variation, and intramuscular fat content would have on the aforementioned chemical changes. A repeat experiment was run after completion of the first one.

Paired beef quarters were obtained from a packing plant immediately after chilling. The three grades, U. S. Prime, Good, and Utility, were chosen to obtain different fat levels. Sections of one quarter of the carcass of each grade were aged at 0°, 35°, and 45°F. for 3 weeks prior to sample preparation and irradiation. The Longissimus dorsi muscle was used and, after being freed from all extraneous fat, was ground, and individual samples were stored after irradiation for 3 months at 35°, 60°, and 90°F. All samples were analyzed for the previously mentioned chemical changes. Gross physical changes, including color and texture, were also recorded. Organoleptic evaluations on selected treatments were made by Dr. A. M. Pearson and associates at Michigan State University.

Results from these experiments indicate certain definite effects by some of the conditions used. Some of the more obvious results are listed below.

(1) Hydrogen sulfide, methyl mercaptan, and acid-soluble carbonyl compounds increased, while glutathione, glycogen, and hydrogen ion concentration decreased with increasing radiation dosage for all grades. These results were consistent in both experiments and with preliminary work.

(2) Pre-aging at higher temperatures (35° and 45°F.) decreased the amount of hydrogen sulfide generated at all dosages, while at the lower holding temperature (0°F.) the amount increased.

(3) Post-irradiation storage for 3 months resulted in decreases in methyl mercaptan. The higher the storage temperature, the greater was the decrease.

(4) Post-irradiation storage resulted in decreased amounts of carbonyl compounds in all grades and storage temperatures, the greatest decrease occurring at the higher temperatures.

(5) In most instances, pre-aging plus irradiation caused complete destruction of glycogen as such.

Organoleptic evaluations indicated no significant difference in taste panel scores between control and irradiated (2 megarads) Prime beef, while the Good and Utility grade controls were significantly more acceptable than their irradiated counterpart. The results obtained by comparing the same meat irradiated at 2 megarads and stored at 60°F. for 3 months to a frozen control (0°F.) indicated the controls were significantly more acceptable than the irradiated samples of all three grades. The acceptability of the controls was also somewhat less than the original.

The odor, which has been designated as "A" odor, was predominant in all grades and aging temperatures irradiated at 2 megarads. At 4 megarads, the "canned dog food" odor occurred along with a darker color, rubbery texture, and increased drip. At 8 megarads, a burnt odor was present with increased dark color, rubbery texture, and drip.

Post-irradiation storage for 3 months at 60°F. and 90°F. produced a barnyard-type odor, with very rubbery texture, excessive drip, and extreme color changes. The surface color of the samples stored at 90°F. was a greenish black.

Although some of the general trends mentioned are obvious, the data are undergoing statistical analysis to determine which variables are significant for the physical and chemical changes which occur.

This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces, QM Research and Engineering Command, U. S. Army, and has been assigned number 849 in the series of papers approved for publication. The views or conclusions contained in this report are those of the author. They are not to be construed as necessarily reflecting the views or indorsement of the Department of Defense.

Effect of Irradiation and Heat upon the Enterococci

By Suzanne Dewees Drake

Division of Bacteriology

Irradiation, or the combination of moderate irradiation plus moderate heat, has been proposed for the processing of canned hams. Knowledge of the heat and radiation resistance of the bacteria that may be in these hams is a necessary preliminary to developing such processes. Much is known about the resistance of spores to heat and irradiation, but relatively little is known about non-sporeforming bacteria.

Certain enterococci, especially Streptococcus faecium, occasionally may be found in virtually pure culture in heat pasteurized canned hams. S. faecium has also been found in minced chicken which has received moderate levels of irradiation. The present study included strains of S. faecalis, S. durans, and S. faecium from canned hams, hog feces, human feces, and from several stock culture collections.

Some strains of S. faecium have been found to be considerably more resistant to both heat and gamma irradiation than are most strains of S. faecalis or most other non-sporeforming bacteria. Most of the strains of S. faecium isolated from canned hams required 1 to 2 hours at 145°F. or 30 to 60 minutes at 150°F. for complete sterilization. The strains of S. faecalis studied were killed in less than an hour at 145°F. or 15 minutes at 150°F. S. durans had a

heat resistance somewhat intermediate between S. faecalis and S. faecium. The most thermal tolerant strains of each species were those which were isolated from canned hams. Those from the intestine were all relatively heat sensitive.

There was no apparent correlation between the radiation resistance and the heat resistance of individual strains although, generally, S. faecium strains were most resistant to radiation, as well as heat. The strains of S. faecium required from 1.7 to 6.5×10^5 rads to kill 99% of the initial population, averaging 3.2×10^5 rads. The dose killing 99% of the initial population averaged 1.4×10^5 for S. faecalis and 2.5×10^5 for S. durans.

One strain of S. faecium which was extremely heat and radiation resistant was chosen for studying the effects of combined heat and radiation. Heating appeared to make this strain slightly more sensitive to radiation. The radiation survival curve, after heating, tended to become exponential rather than the multi-hit type of curve which was obtained with the unheated bacteria. However, after the initial lag, the curves were parallel, indicating that this was not an actual increase in radiation sensitivity.

However, radiation did tend to sensitize this streptococcus to subsequent heating. There was not only a decrease or elimination of the initial lag but an increase in the slope of the survivor curves. This would suggest that moderate radiation followed by moderate heat might prove to be a feasible method for processing canned hams.

This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces, QM Research and Engineering Command, U.S. Army, and has been assigned number 868 in the series of papers approved for publication. The views or conclusions contained in this report are those of the author. They are not to be construed as necessarily reflecting the views or indorsement of the Department of Defense.

Pedilococcus cerevisiae as a Starter Culture for Pork Roll and Lebanon Bologna

By G. D. Wilson

Division of Food Technology

In the past year we have extended our studies on the use of the starter culture to types of fermented sausages other than that of the summer sausage type. We believe that a starter culture can be employed advantageously in all kinds of fermented sausage, but each has its peculiarities. For this reason, it could not be assumed that the results obtained for summer sausage could be applied directly to pork roll, Lebanon bologna, or dry sausage. The primary emphasis has been on pork roll and Lebanon bologna, but our studies on dry sausage are underway.

Pork roll and Lebanon bologna are found most frequently in Eastern markets and may not be familiar to those whose trade is confined to the Midwest or West. We believe, however, that pork roll in particular would find acceptance in these markets.

Our experimental work with pork roll has centered about the establishment of a formula and processing schedule which will produce a product comparable to commercial products with the attendant benefits provided by the use of a starter culture. Commercial pork roll is an all-pork product containing about 33% fat, 42% moisture, and 18% protein, by analysis.

Experiments showed that a pork roll with the general



Pork roll produced in AMIF sausage kitchen is inspected by Frank Mills of Division of Food Technology during studies to provide information on use of starter culture in this type of meat product.

flavor, texture, color, and appearance of commercial products could be produced in 40 to 48 hours using the starter culture. It was further demonstrated that the flavor of the product could be altered through variations in sugar, salt, and seasoning and that a desired flavor was reproducible.

Commercial Lebanon bologna is an all-beef product which has a characteristic flavor which presumably is developed during an aging procedure prior to the manufacture of the sausage. The first experimental attempts to reproduce commercial products with the starter culture using unaged beef resulted in a tangy sausage, but the desired characteristic flavor was absent. Experiments which followed allowed for aging of the beef prior to use. Following commercial practice, the beef was cut into one pound pieces and aged in 3.0% salt at 45°F. These tests indicated that, depending on the original condition of the beef, from 3 to 6 days of aging was required to develop the characteristic flavor in the finished product. Further investigation is needed to determine the nature of the changes during aging which produce a "Lebanon flavor." A fuller understanding of these changes would provide an opportunity for controlling this phase of production, as well as the fermentation which takes place in the heat processing.

The studies on dry sausage have been initiated using Genoa salami as the experimental product. Products now under test have been prepared with the starter culture and subjected to various degrees of fermentation before being placed in the drying room. Drying rates under controlled conditions of temperature, humidity, and air velocity are being followed in order to develop more accurate information on drying characteristics of this type of product. The sausage made with the starter culture has a good general appearance. Further evaluation will be made after the minimum drying time of 45 days.

In summary, we have demonstrated the application of a starter culture in pork roll and Lebanon bologna. In the case of Lebanon bologna, the changes which take place prior to fermentation need to be clarified to bring the production of this product under quality control. Studies of the application of the starter culture to dry sausage are encouraging and promise to yield much needed information concerning the drying process.

This research was supported in part by a grant from Merck & Co., Inc., Rahway, New Jersey.

A Re-evaluation of the Role of Sugar in the Curing of Hams

By Franklin Mills

Division of Food Technology

Several functions for the role of sugar in the curing of hams have been proposed. Principal among them are: (a) the enhancement or development of flavor, (b) aid in the development and protection of the cured meat color, and (c) possible retardation of a putrefactive type of spoilage. Sucrose is the sugar used most extensively in curing meat.

In order to evaluate the role of sugar in the curing of hams, a study was instituted to compare sucrose and dextrose with non-caloric sweeteners (calcium and sodium cyclamate). Twenty-four pairs of hams were randomized, with respect to sides and cure, and artery pumped to 108% of their green weight. The pump pickles were made with each of the sweetening agents, sucrose, dextrose, Ca-cyclamate, and Na-cyclamate. These substances were added to the pickles on the same sweetening basis as sucrose, which was added at a level of 25 pounds per 100 gallons. In addition to these pickles, one pickle had no sweetening agent, and one pickle contained twice the level of sucrose as used in the normal sucrose pickle. The hams were placed in evacuated plastic bags, cured for 7 days at 40°F., and then processed in the smokehouse to an internal temperature of 155°F.

Since the final experiments were completed only

recently, a statistical analysis of all the data has not been completed and the results reported here are those from taste panels and from objective color analyses.

The taste panel analysis was designed to compare each sweetening agent with all others. A panel of 7 to 9 members was asked to distinguish between samples in a triangle test and to state their bases for distinction: flavor, texture, color, odor, etc. Most of the selections were based on flavor. Results of the taste panel evaluation are summarized in Table 1. Judges were able to detect a significant difference between the hams when they compared those cured without sugar and those cured with twice the normal amount of sugar.

Table 1

Taste Panel Results for Sweetening Agents in Hams

<u>Treatment</u>	<u>No. of Judgments</u>	<u>Correct Judgments</u>	<u>Signifi- cance</u>
Ca-Cycl. vs. No Sugar	36	14	
Ca-Cycl. vs. Sucrose	32	9	
Ca-Cycl. vs. Dextrose	34	15	
Ca-Cycl. vs. Na-Cycl.	36	12	
Na-Cycl. vs. No Sugar	36	16	
Na-Cycl. vs. Sucrose	36	16	
Na-Cycl. vs. Dextrose	34	17	P=0.05
Dextrose vs. No Sugar	32	14	
Dextrose vs. Sucrose	18	9	
Sucrose vs. No Sugar	36	12	
Sucrose vs. 2X Sucrose	52	29	P=0.001

A flavor difference was noted also between a ham cured with Na-cyclamate and with dextrose, but this difference was probably due to judgments of one substitute

panel member who was always able to detect the hams cured with cyclamates. These judgments probably reflect a low threshold by this one judge and suggest the possibility that a portion of the general population may have a similar threshold. Information on the distribution of this characteristic is required before conclusions on the effect of cyclamates on flavor can be drawn. Additional experiments using other concentrations of these sweetening agents may establish flavor differences not detected in this study.

The spectral analysis of the color of the hams was made with the Beckman DK-2 recording spectrophotometer on acetone extracts of the ham samples. A preliminary statistical analysis of the data so far obtained indicates that there is no difference in the effects of treatments upon the production of the cured meat color. In all cases there was a significant fading of the cured color upon refrigerated exposure to light. However, there was no significant variation between the treatments and the rate of color fading, thus indicating no effect of any one treatment upon the amount of fading.

In the light of these data, it would seem that sugar in ham curing is not as important in today's modern meat packing practices as it might have been in the past.

This research was supported in part by a grant from Abbott Laboratories, North Chicago, Illinois.

Techniques for Studying Water Balance in Animal Tissues

By Matthew C. Urbin

Division of Food Technology

During the past year the Foundation has undertaken a project designed to study the water binding capacity of meat. Before beginning this work, we were confronted by the problem of not knowing the variations which exist in the distribution of water between the intracellular and extracellular phases either at the time of slaughter or what occurs after slaughter. Since the distribution of water can greatly influence the water binding capacity of meat, it was decided to investigate this aspect before beginning work on protein binding of water.

All methods for the determination of the extracellular-intracellular ratio of water are dilution type techniques. Either they take advantage of some constituent which normally is found totally in the extracellular phase or they depend upon the injection of some material which will distribute itself according to the water spaces. Chloride ion is an example of the former, while inulin or thiosulfate is an example of the latter. The work which will be described here involves the measurement of chloride space and inulin space.

The chloride method has been used for those experiments in which the animal was to be sacrificed and distribution was to be determined at the time of kill. Essentially, this method involves a measurement of the blood chloride



Determination of variations in intracellular and extracellular distribution of water in muscle tissue constitutes an important preliminary phase of AMIF study of water binding capacity of meat. Laboratory rats, raised on varying diets and to varying ages, were sacrificed to permit D. J. Ginsberg and M. C. Urbin of Food Technology to compile essential data.

level and the muscle chloride level, and calculating the intracellular-extracellular ratio from these two measurements. The method has been used on rats which have had different fats in their diets and were sacrificed when they were one year old. Differences were found, depending primarily on the type of fat in the diet. Variations in age of rats were also followed in this manner.

Inulin is used in live animals to measure extracellular water; Evans blue can be used to measure the blood volume; while antipyrine is distributed throughout all body water and can be used as a measure of total body water. This portion of the work thus far has been a matter of establishing methodology. Using the rabbit as an experimental animal, this method has proved rather satisfactory. It is hoped that this method can be adapted to excised muscle for the purpose of following changes.

BEEF TENDERNESS STUDIES

Studies on the Proteolytic Enzymes in Beef Muscle

By R. A. Sliwinski

Division of Analytical and Physical Chemistry

The fact that meat undergoes physical and chemical changes during storage has long been observed. Increased tenderness and flavor changes characterize meat which has been stored for some time. Some of the chemical changes can be attributed to the presence of bacterial flora, but many have not been completely explained except for the suggestion that they are brought about by the action of enzymes.

Very little is known about the proteolytic enzymes in muscle. Information regarding these enzymes in muscle tissue, which degrade the protein molecule into smaller units, polypeptides, and amino acids, would be of considerable basic interest in itself besides being of practical value to the meat industry with respect to the problem of meat tenderization. This knowledge may, and should, answer some of the questions pertinent to the mechanism of the physical and chemical changes which occur during the storage of meat.

The study of these entities inherent in beef muscle tissue has been undertaken at the Foundation. These studies were initiated to determine, isolate, and define the proteolytic enzymes which are present per se in beef muscle.

Previous work had shown that a simple and sensitive method for determining total proteolysis in meat or meat

extracts was needed. A method was developed whereby water extracts of fresh meat were incubated with a buffered hemoglobin substrate and the index of proteolytic activity was determined by spectrophotometrically measuring, at a wavelength of 276 mμ, the amount of trichloroacetic acid soluble tyrosine liberated from the substrate.

Preliminary experiments showed that extraneous materials which absorbed in the range of tyrosine were extracted from the meat along with the enzymes and thereby interfered with the assay. The removal of these unwanted absorbing substances was attempted. It was found that, after the meat was extracted, dialysis of the extracts prior to the assay of the enzymes almost completely removed the extraneous absorbing substances without loss of proteolytic activity.

The data from these experiments indicate that proteolytic activity increases after dialysis of the meat extracts prior to the assay, and the activity was greater when the meat was extracted at a higher pH.

Information has been obtained regarding the optimal conditions of temperature, incubation time, pH, enzyme concentrations, etc. Using denatured hemoglobin as the substrate whereby the final concentration of the reaction mixture was 1% hemoglobin in 0.1M acetate buffer, the optimum conditions of the assay were found to be at a pH of 4.4 if incubated for 4 hours at 37°C. Evidence from the kinetic experiments indicates that a number of enzymes are involved in the over-all proteolysis.

Various extractions and isolation procedures were investigated and, at the present time, active concentrates of the enzymes have been obtained from half

saturated ammonium sulfate fractions. Suitable methods of separating and isolating the various types of activity from these concentrates are being studied. The exact number and specific nature of the individual enzymes involved in muscle proteolysis remains, at the present time, a question yet to be answered.



Marion L. Birkner of Histology Division makes microscopic measurement of diameter and extensibility of isolated muscle fibers from raw and cooked beef during studies to determine role of salt in tenderization of meat. This is one phase of extensive AMIF research on tenderization.

Action of Sodium Chloride upon Cooked and Raw Isolated Muscle Fibers

By Marion L. Birkner

Division of Histology

Much interest recently has been expressed in the role of salts in muscle protein hydration. Histological observations of such treated tissues are limited. Tofte (Thesis, Iowa State College, 1940) reported muscle fibers were much swollen and had indistinct outlines when observed in the raw, salt-treated state. Cooking, with its concomitant loss of weight, apparently restored the fibers to their normal size and appearance of individuality. Suri (Fleischwirtschaft, 10, 622, 1957) reported damage to the connective tissue and shrinkage of the muscle fiber bundles in both raw and cooked samples infused with sodium chloride.

Recent Foundation reports have pointed to an increased tenderness of frozen-dehydrated steaks rehydrated in salt. This, plus the fact that many commercially available tenderizers contain varying percentages of salt, prompted a survey of structural changes of muscle fibers brought about by sodium chloride.

Isolated muscle fibers of raw and cooked round were obtained by blending and were directly immersed in 2, 7, and 14% sodium chloride solutions. The diameter and extensibility measurements were expressed as percentage of change. With all three solutions employed, there was a rapid initial rate of diameter increase

within 15 minutes. A solution of 2% salt produced 30% increase, 7% salt a 45% increase, and 14% salt resulted in an intermediate value of 40%. No appreciable effect on the diameters of pre-cooked or heat denatured fibers was observed.

Extensibility measurements were made on similarly treated fibers at various time intervals. A 5 mm. length of each fiber was stretched to the breaking point. Average length at the time of breakage was expressed as the percentage of increase over that of the control.

Extensibility has been previously shown to bear an inverse relation to tenderness (Wang, et al., J. Animal Sci., 15, 97, 1956). In this case, only the 2% sodium chloride reduced the extensibility, indicating a less elastic or more tender fiber. Seven and 14% solutions produced 160 and 120% extensibility, respectively, in 15 minutes. After 90 minutes of immersion, these fibers still exceeded values for the controls. Thus, the physical property of extensibility (and thus possibly tenderness) does not appear to be related to the increased hydration of isolated muscle fibers as measured by diameter increase.

In contrast to these observations on isolated muscle fibers, fibers of salt-treated steaks (both fresh and dehydrated) revealed no significant increase in fiber diameter. Neither were any structural changes apparent in raw frozen-dehydrated Longissimus dorsi steaks rehydrated in 2% sodium chloride solution. Salt rehydration followed by cooking, however, did produce loss of muscle fiber structure or integrity. This lack of fiber diameter increase and the obvious structural degradation indicate that one role of salt in tenderization is that of protein solubility.

Effect of Enzyme Combinations upon Tenderness and Residue of Freeze-Dried Beef

By C. Edith Weir

Division of Home Economics

The cooperative program of histologic and panel techniques in progress at the American Meat Institute Foundation has been successful in furthering our knowledge of the underlying causes of tough or tender meat. Several enzymes have been shown to affect both muscle structure and tenderness, although their mode of action upon the muscle and total effect upon tenderness are not the same. Fungal amylase and ficin break down the muscle fiber substance; rhozyme and papain have less effect: and bromelin only a slight effect. All of the enzymes increased the initial tenderness of the meat. Bromelin, ficin, and papain cause disintegration of both collagenous and elastic connective tissue, while amylase shows a trace of collagenous activity, and rhozyme none. Those enzymes acting upon the connective tissue also decrease the amount of residue left after chewing.

Preliminary studies indicate that the manner in which combinations of the enzymes attack the muscle is dissimilar to the action of the enzymes used singly. For these studies, dehydro-frozen steaks prepared from the Semitendinosus muscle of Utility grade beef carcasses were rehydrated in aqueous enzyme solutions for histologic study, and in 2% sodium chloride solutions containing enzymes, followed by broiling, for panel evaluations. Amylase, bromelin, ficin, papain, and Rhozyme P-11,



Steaks prepared for taste-panel evaluation of tenderness are grilled to precise internal temperatures to insure identical degree of cook. Here, Betty Ann Ginger of Home Economics places enzyme-treated steak in broiler. Thermocouple in steak permits exact temperature reading.

combined in pairs, brought about different changes in actomysin and elastin than when the enzymes were used singly.

Interpretation of the results is made difficult by the inability to relate enzyme concentration to extent of either histological change or panel estimates of tenderness, i. e., 0.0002% of an enzyme may produce either more or less than twice the effect of 0.0001% of the enzyme. This was demonstrated by data obtained by panel evaluation of initial tenderness and residue in steaks treated with bromelin and papain. Five adjoining steaks from each of three muscles were used for every test series. Steaks 1 and 5 were rehydrated in 2% sodium chloride solution and served as controls. Bromelin and papain at a concentration of 0.0001% produced a slight increase in tenderness, but together they resulted in a slight increase over the sum of each used singly. However, when the total enzyme concentration was 0.0002%, either bromelin or papain was more effective than when they were combined in equal amounts. It would seem, therefore, that the greater effect produced by the enzymes used together was the result of concentration rather than synergism.

Bromelin and papain concentrations of 0.0003% have been found to produce the most satisfactory improvement in meat tenderness. The addition of 0.0001% of either enzyme to 0.0003% of the other resulted in an increase in tenderness about equal to the sum of the effects caused by each enzyme alone.

The use of combinations of papain and bromelin at low levels had no advantage over the same total quantity of either used alone. At higher levels of concentration, the addition of 0.0001% of papain or bromelin to 0.0003% of the other increased the tenderness of the meat,

but it is not known whether increasing the total quantity of either enzyme to 0.0004% might not be as effective.

The effects of different amounts of papain and bromelin upon residue were similar to those upon tenderness. The effect of either 0.0001% bromelin or papain was very slight and was augmented by the presence of either 0.0001% or 0.0003% of the other. The effect was especially prominent when 0.0001% bromelin was added to 0.0003% papain.

It has been proposed in other reports from these laboratories that tenderness changes induced by the crude enzyme preparations used in our studies were the result of more than one specific enzyme. The studies performed to date using enzyme combinations suggest that in papain and bromelin preparations these constituents may not be identical.

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Further Studies on Use of Elevated Temperatures to Increase the Tenderness of Beef

By Paul D. Brown

Division of Food Technology

In our studies on high temperature beef aging, the objective is to establish an optimum aging time and temperature for the rapid tenderization of beef consistent with suitable microflora control. Experiments have been conducted to determine tenderness increases due to aging at elevated temperatures, the effects of gamma irradiation on tenderness, and the effects of high temperature aging as compared to a more conventional aging procedure.

The beef used in these studies was 3/4 inch steaks, cut from either U. S. Utility or U. S. Good inside rounds, which previously had been stitch pumped with an antibiotic solution. The Semimembranosus muscle was dissected out of the steaks, vacuum packaged, and allotted to an aging treatment.

The experimental design allowed for randomization of aging treatments between adjacent steaks of the same round. Sampling in this manner was deemed necessary since studies of the tenderness characteristics along the Semimembranosus muscle indicated rather large initial differences between steaks. Tenderness of the broiled steaks was evaluated by an eight member taste panel and by a Warner-Bratzler shear machine.

Some initial experiments using gamma radiation as



Beef steaks, experimentally treated to prevent spoilage, are placed in controlled-temperature cabinet by Paul D. Brown of Food Technology during study of tenderization by high-temperature aging.

a preservative suggested that irradiation might inhibit tenderization of the steaks during the aging period. An experiment was designed to study this apparent effect. Matched inside rounds from eight U. S. Good grade carcasses were infused with oxytetracycline and cut into steaks. The steaks which were irradiated received 45.5 thousand rads. Steaks were either unaged or aged 24 hours at 110°F. and, after a bacteriological analysis, were evaluated for tenderness. An analysis of the results indicated no effect on tenderness at the level of irradiation used. The analysis did, however, indicate a significant increase in tenderness after aging for 24 hours at 110°F., which supports the findings of other experiments.

Another experiment was designed to determine the optimum aging time for tenderization at 110°F. For this study, eight pairs of rounds were infused with oxytetracycline and cut into steaks which were allotted to an aging time of 0, 16, 24, or 40 hours at 35°F. or 110°F. After aging and bacteriological analysis, the steaks were submitted to the taste panel and were shear tested. Significant increases in tenderness were obtained by aging steaks at 110°F. for 24 hours and no additional increase occurred when steaks were aged 40 hours.

It was also desirable to compare high temperature aging to a more conventional aging procedure with respect to tenderization. An experiment was designed to compare steaks aged 24 hours at 110°F. to steaks aged 14 days at 35°F. The steaks were cut from eight pairs of inside rounds which had been infused with oxytetracycline. After packaging, the steaks were allotted to one of the aging treatments mentioned above or frozen immediately as unaged controls. The steaks aged at 110°F. for 24 hours were as tender as the steaks aged at 35°F. for 14 days. It would be desirable to determine precisely

the most effective high temperature for tenderization and whether the same relationship between aging treatments holds true when wholesale cuts are aged.

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